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The effect of mechanical constraints on gelatin samples under pulsatile flux

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Abstract. It is of great interest in tissue engineering the role of collagen gel-based structures (scaffolds, grafts and -by cell seeded and maturation- tissue equivalents (TEs) for several purposes). It is expected the appropriate biological compatibility when the extracellular matrix (ECM) is collagen-based. Regarding the mechanical properties (MP), great efforts in tissue engineering are focused in tailoring TE properties by controlling ECM composition and organization. When cells are seeded, the collagen network is remodeled by cell-driven compaction and consolidation, produced mainly through the mechanical stimuli that can be directed selecting the geometry and the surfaces exposed to the cells. Collagen gels have different (chemical and mechanical) properties depending on their origin and preparation conditions. The MP of the collagen network are derived from the degree of cross-linking (CLD) which can be modified by different treatments. One of the techniques to evaluate MP in the network is by ultrasound (US). In this work we analyse the effect of several mechanical constraints (similar to that imposed to promote cell growth on certain sample surfaces, when seeded) on samples of gelatin with a specific geometry (thick walls cylinders) under loading conditions of pulsatile flow. We checked US parameters and estimates evolution of the network structure for different restrictions in the sample mobility. It was implemented by adapting devices specially built to measure elastic properties of biological tissues by US. The material (origin and purity) and the preparation conditions for the gelatin were selected in order to compare the results with those of literature.

Introduction

Collagen-based hydrogels became interesting in a wide variety of applications, like medicine, pharmacology, cosmetics, foods, photography, packaging ([1] and references therein).

In particular, gelatin (a polypeptide derived by hydrolytic degradation of collagen [2]) have gained importance due to several factors, some of them are: its abundance in the nature, renewability, non-toxicity and bio-degradability. Collagen is the primary structural protein in mammals, as constituent of the major part of the extracellular matrices in connective tissue. Its biological function is largely, if not exclusively, mechanical [3]. Processing characteristics of collagen based hydrogels made them attractive, in particular the "tunability" of their mechanical properties for applicative purposes [1], which is achieved by different preparation, concentration, bakery, congelation temperature and time, stirring, aging, addition of crosslinkers, application of radiation doses or addition of different substances [4-6]. The network structure and the physical properties of the gelatin gels are mainly conditioned by the source and the conditions of extraction [1]. Crosslinking improves mechanical and thermal stability of the gelatin [1]. Changes in the crosslink can be promote by chemical substances (the most common is glutaraldehyde [1]), electromagnetic radiation (among others, UV-rays [7]) and cellular work when seeded to build tissue equivalent (TE) in tissue engineering [8].

Collagen hydrogels provide an in-vivo-like 3D environment to seed cells to prepare TE [8]. Remodeling as consequence of the cellular activity is a topic of permanent research [8 and references therein]. The mechanical properties of the hydrogels can be tested by several ways. Among the indirect tests to investigate them, ultrasound (US) interrogation has several interesting characteristics: non destructiveness, non ionizing radiation and -depending on the device- the possibility of monitoring the evolution of the sample in the place of interest (as in a bioreactor or when implanted). Special care must be taken with the results interpretation, because -even in the case of conventional direct tests- they are strongly dependent on the model selected for the material, the test conditions and the geometry and dimensions of the samples [9].
In this paper we present some results that are part of the study of the response of gelatine thick walled tubes under loading conditions of internal pulsatile flow. In the following paragraphs we describe the preparation of the samples and the experimental setup. Two type of restrictions on the movement of the gelatine tubes were imposed. The results are commented.

Materials and Methods

Sample preparation. Ten percent weight in volume of gelatin powder from bovine type B was hydrated with a solution of deionized water and heated above 70°C to disperse the colloid, clarify the solution, and release trapped gases. Care was taken to prevent introducing air while mixing. Cross-linking among collagen fibers occurs when the mixture is sufficiently cold (approximately 26°C for gelatin). The emulsion was cooled to 30°C before being poured into the cylindrical molds overnight. The mold consisted in two concentric tubes (see Fig. 1 (b)), the one of the exterior (acrylic) with an internal diameter of d_3=14.94mm (SD 0.05mm) and the interior tube (silicone) with an external diameter of 6.88mm (SD 0.02mm) and a wall thickness of 0.94mm (SD 0.02mm), being the mean wall thickness of the gelatine cylinder 4.03mm at rest. Its longitude was 6cm.
Together with the tubular samples, gelatin cylindrical samples were prepared in a multiwell in order to check speed of sound at the same experimental conditions.

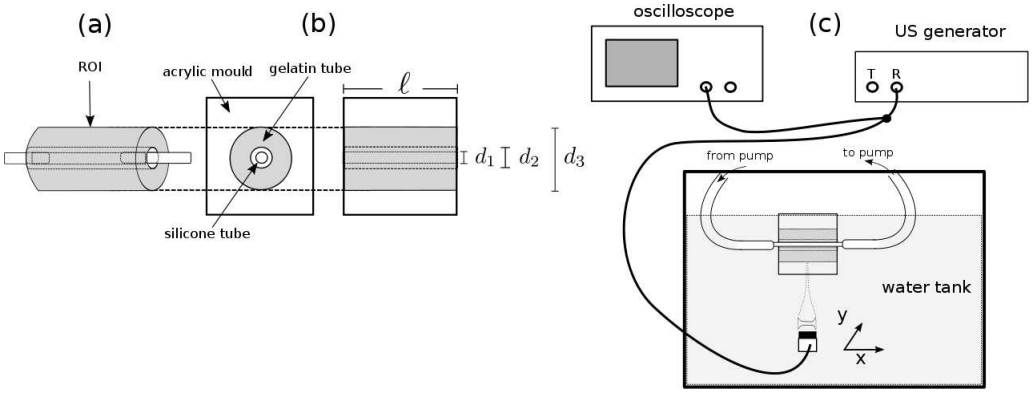

Figure 1. (a) Unconfined gelatin tube sample. (b) Acrylic container with gelatin in a silicon tube. (c) Experimental setup.

Experiments. The experiments were conducted in a distilled water tank at room temperature (T=23ºC). A block diagram of the experimental setup is shown in Fig. 1 (c). A 4 MHz ultrasound transducer (General Electric Z4K) was used in pulse / echo mode. The center of the sample was placed by the controlled arm (see x, y axis in Fig. 1 (c)) at the focal plane of the transducer and the axis of the ultrasonic beam was set normal to the cylinder axis. A Kraut Kramer USN 60 ultrasound generator was used together with a digital oscilloscope Tektronix TDS 210 to acquire the signals. A personal computer and user built Matlab routines were used to processing the data. As it is shown in Fig. 1 (c) peristaltic pump was connected to the cylindrical sample in such a way that water flows in the internal flexible silicone tube.

Two different experiments were conducted. The former was performed by pumping in the samples within the acrylic mould (experiment 1). In this way the sample was under confined conditions. The second one, pumping was done with the samples without mould; as a result a no confined condition was obtained (experiment 2).

Signal processing. Speed of sound is computed by calculating the time of fly (TOF) of the signal in the medium under test. This is done by recognizing the echoes of each layer of known thickness. In this work, TOFs were computed by applying the continuous wavelet transform (CWT) to each RF-signal. The CWT is defined by Eq.1:

$$L_{\Psi} f(a,b) = c \int_{-\infty}^{\infty} f(t) \Psi^{*}_{a,b}(t) dt \tag{1}$$

where $f(t)$ is the RF-signal, $\Psi^{*}_{a,b}(t)$ is the complex conjugate of the wavelet, c is a wavelet-specific constant and $L_{\Psi} f(a,b)$ is the transformed RF-signal corresponding to a and b.

The wavelet $\Psi^{*}_{a,b}(t)$ is derived from a mother-wavelet $\Psi(t)$ (in this paper the so called Mexican hat is used), which can be shifted in time by the variable b and can be scaled (stretched or compressed) by the variable a:

$$\Psi_{a,b}(t) = |a|^{1/2} \Psi\left(\frac{t-b}{a}\right) \tag{2}$$

The result of the transform is a matrix of coefficients depending on time (or space, b) and scale (a) (related to the frequency). The maximum indicates when the maximum power in a particular frequency (scale) arrives at a particular time to the transducer. Detecting the maximum of the transformed signal its TOF was determined and SOS was calculated.

An example of experiment 1 measurement is shown in Fig.2. The media under measurement is the acrylic mould with the gelatin tube. In Fig. 2 (a) is shown the RF signal. Figure 2 (b) shows the absolute values of wavelet coefficients in grey scale. The more white the higher the absolute value of the coefficient. As can be seen each layer reflection is marked with an arrow: water/acrylic, acrylic/gelatin, gelatin/silicone wall 1, gelatin/silicone wall 2 (interior), etc. Obviously, the scale with the highest power is the equivalent to 4MHz, this scale is chosen to calculate the TOF in gelatin.

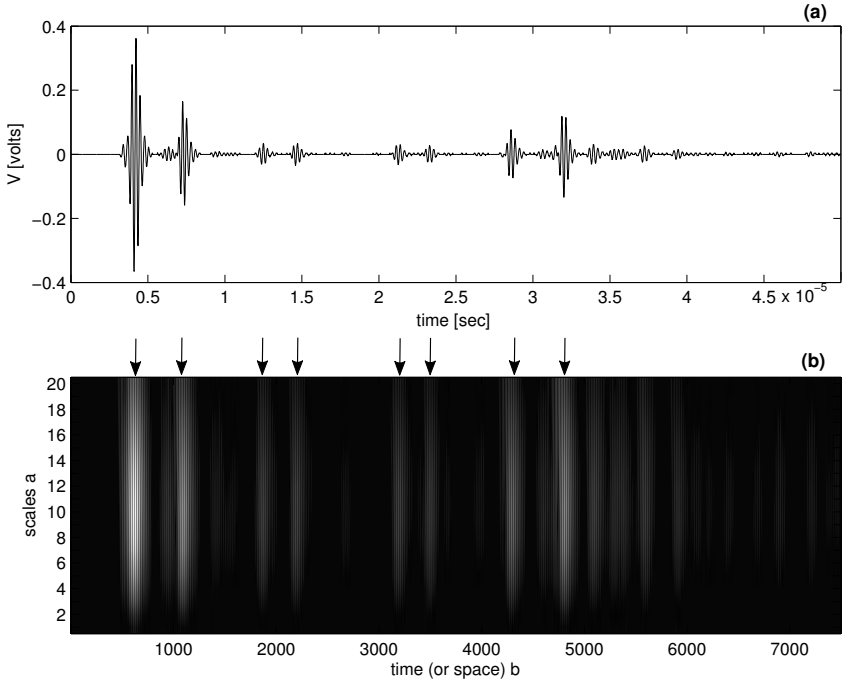

Figure 2. Experiment 1. Acrylic container with gelatin in a silicon tube. (a) Typical RF signal. (b) Absolute values of wavelet coefficients using a Mexican hat. The arrows indicate the different interfaces.

The difficulty in the experiment 2 is that the pumping moves the gelatin tube; therefore, a cross correlation technique must be implemented to align the signals (from different time instants) using the first echo of signal 1 as reference (see Fig.3 (a-b)). In this way, a tracking of the wall gelatin thickness could be implemented under the supposition that speed of sound is constant in the gelatin. In Fig. 3 (c) the delayed or advanced time τ (positive or negative, respectively) obtained by cross correlation is plotted for four signals aligned with the first one.

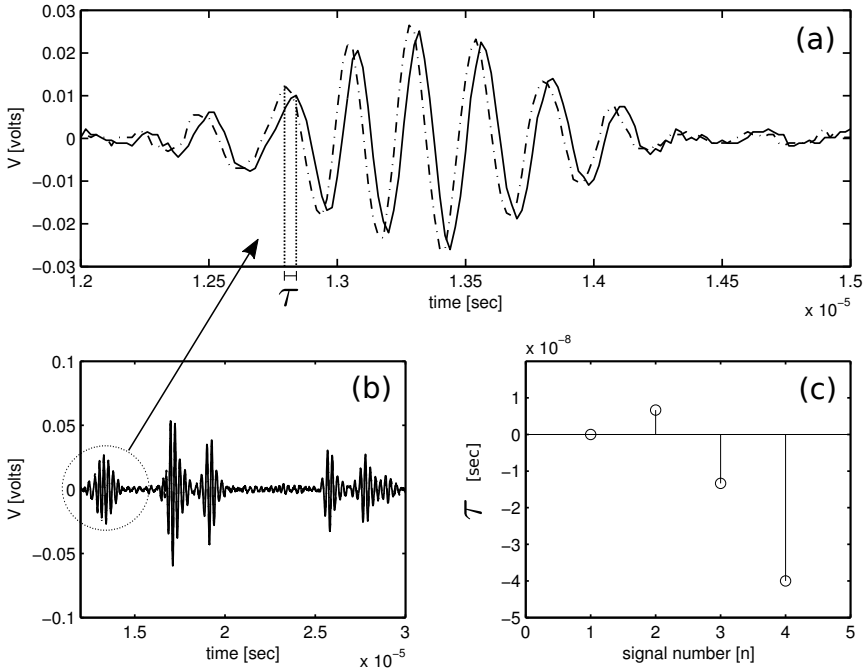

Figure 3. Experiment 2. Alignment using cross correlation technique. (a) First echoes of signals 1 (dashed line) and 2 (continuous line). (b) RF signal from water/gelatin to water/silicone wall tube. (c) τ obtained by cross correlation.

Results

As it was mentioned above, gelatin samples were prepared in a multiwell with the same gelatin solution that was used to prepare the gelatin tubes. They were measured within the multiwell in the water tank at the same experimental conditions. The obtained speed of sound was 1532.4±0.1 m/sec (mean value ± standard deviation). It is remarkable that for each sample 128 RF signals were averaged.

Before starting the pump 128 RF signals were averaged for both experiment 1 and 2 (confined and unconfined), therefore two estimates of the wall thickness by ultrasound were obtained 4.011mm and 4.088mm, respectively. Figure 4 shows the tracking of the wall gelatin tube thickness taking four different signals in experiments 1 and 2 (acquired between arbitrary chosen intervals of time) while the pumping process started. In this figure the speed of sound was considered constant in the gelatin, as a consequence, the wall thickness can be estimated. TOF of signals were calculated using the procedure explained above.

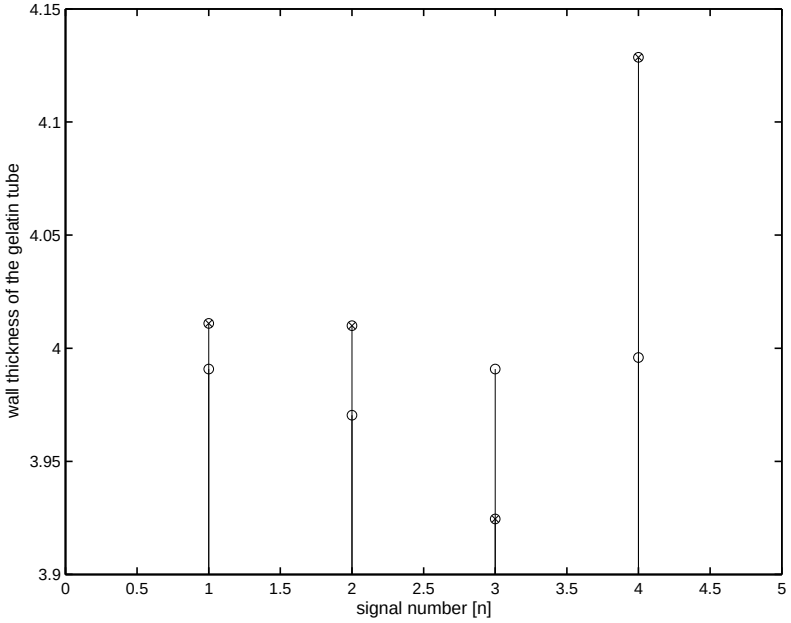

Figure 4. Wall thickness tracking of the gelatin tube in unconfined (circle) and confined (circle with a "x") condition during pumping.

Discussions and Conclusions

In both experiments, the obtained value for the wall thickness falls in the range of expected values, being , in the case of unconfined condition, within the range of the thickness at rest. For the unconfined condition, the value exceeds in approximately 20% the value of the wall thickness at rest. This effect does not proceed from water absorption, because the samples were left enough time in the water tank for degassing and stabilizing the temperature before connecting the pump.

Supposing the speed of sound is the same in the three cases (1532m/sec), wall thickness can be estimated with high accuracy for this flow conditions. On the other hand, if wall thickness is estimated by an independent method, an estimate of the speed of sound can be obtained; therefore the mechanical properties could be assessed. This last computation is being analyzed together with the mathematical model for the material to extract its mechanical properties.

The wavelet transform is a novel approach to detect the time of fly of a roughly surface as a construct plus cells, because the main idea of the method is to detect when the maximum power arrives to the transducer, avoiding problems like thresholds definitions.

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